abcam

Product datasheet

Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free ab219587

Recombinant RabMAb

24 References 7 Images

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Product name Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free

Description Rabbit monoclonal [EP786Y] to ROCK2 + ROCK1 - BSA and Azide free

Host species Rabbit

Specificity This antibody recognizes both the cleaved C-terminus of ROCK 1 (30 kDa) and full length protein

(158 kDa). The immunogen used for this product shares 83% homology with ROCK2 and has been shown to bind recombinant human ROCK2, please see western blot images below.

Tested applications Suitable for: IHC-Fr, WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Cow, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Untreated, Calyculin A treated and Camptothecin treated HeLa cell lysates. Jurkat, Ramos,

PC-12 and RAW264.7 cell lysates. IHC-P: Human adenocarcinoma of the colon and thyroid gland

carcinoma tissues. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa cell lysate.

General notes ab219587 is the carrier-free version of <u>ab45171</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply

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- Animal-free production

For more information see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEP786Y

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab219587 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration. PubMed: 19295659
WB		Use at an assay dependent concentration. Predicted molecular weight: 158 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

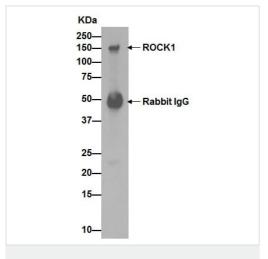
Target

Cellular localization

ROCK2: Cytoplasm. Cell membrane. Cytoplasmic, and associated with actin microfilaments and the plasma membrane. ROCK1: Cytoplasm. Cytoplasm, cytoskeleton, microtubule organizing

center, centrosome, centriole. Golgi apparatus membrane. Cell projection, bleb. Cytoplasm, cytoskeleton. Cell membrane. Cell projection, lamellipodium. Cell projection, ruffle. Associated with the mother centriole and an intercentriolar linker. Colocalizes with ITGB1BP1 and ITGB1 at the cell membrane predominantly in lamellipodia and membrane ruffles, but also in retraction fibers. Localizes at the cell membrane in an ITGB1BP1-dependent manner (By similarity). A small proportion is associated with Golgi membranes.

Images



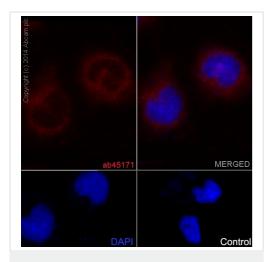
Immunoprecipitation - Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

<u>ab45171</u> (purified) at 1/40 immunoprecipitating ROCK2 + ROCK1 in HeLa cell lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45171).

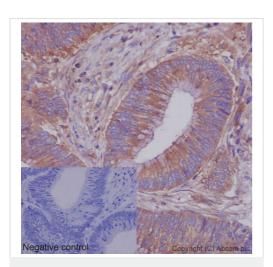


Immunocytochemistry/ Immunofluorescence - Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ROCK2 + ROCK1 with purified <u>ab45171</u> at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <u>ab150078</u>, an Alexa Fluor[®] 555-conjugated goat antirabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/50) and secondary antibody, **ab150113**, an Alexa Fluor[®] 488-conjugated goat anti-mouse IgG (1/500).

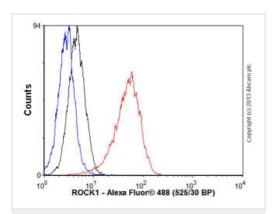
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45171).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of the colon tissue labelling ROCK2 + ROCK1 with purified **ab45171** at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

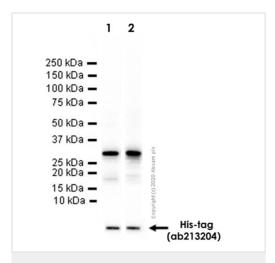
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45171).



Flow Cytometry (Intracellular) - Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

Overlay histogram showing HeLa cells stained with unpurified <u>ab45171</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (<u>ab45171</u>, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor[®] 488 goat anti-rabbit lgG (H+L) (<u>ab150077</u>) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45171).



Western blot - Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

All lanes : Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171) at 1/1000 dilution

Lane 1 : Recombinant Human ROCK1 protein (aa 1114 to 1354) (30 kDa)

Lane 2: Recombinant Human ROCK2 protein (aa 1132 to 1388) (30 kDa)

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 158 kDa **Observed band size:** 30 kDa

This data was developed using <u>ab45171</u>, the same antibody clone in a different buffer formulation.

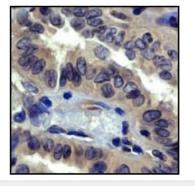
Loading control: Anti-6X His tag® antibody [EPR20547] (ab213204)

Blocking buffer and concentration: 5% NFDM/TBST

Exposure Times:

Lane 1: 3 seconds

Lane 2: 7 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid gland carcinoma tissue labelling ROCK2 + ROCK1 with unpurified <u>ab45171</u>.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab45171).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-ROCK2 + ROCK1 antibody [EP786Y] - BSA and Azide free (ab219587)

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